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Critical feasibility concerns, and therefore phase I objectives, are: i) transfer of the technology from an academic lab (JILA) to industry (Vescent), ii) thin film characterization and definition of a manufacturing protocol to provide reproducible SERS active sites, and iii) demonstration of the Raman enhancement via recording the spectrum of a known analyte. All of the phase I objectives were met. Vescent Photonics worked within the JILA laboratory in order to learn both the synthesis techniques and the metrology methodology associated with photo-generated SERS films. This experience facilitated the design of a Vescent experimental apparatus, which enabled production and analysis of SERS films at the Vescent facility. This was one of the key objectives for technology transfer from JILA ato industry. The polymer films were scrutinized by stylus profilometry, and atomic force microscopy. The process tolerances for uniform film generation were refined, and most notably, the film thickness was determined to be <10nm. This is qualitatively different than previous estimates of $=100$nm. Parameter space was explored and a manufacturing protocol was established for reporducible manufacturing of these ultra-thin films. A final objective of this phase I contract was to record the SERS spectrum of a known analayte. Multiple techniques of exposing the SERS-active to known analytes were explored. The best results wer obtained via doping of analytes into the polymer film, which yielded spectral features for trans 1-2 bis (3-pyridyl) enhylene (BPE) and toluene. The time resolved nature of these spectra (e.g. blinking) is strongly suggestive of single molecule behavior.

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Transmittal Letter for Final Report

A report of Vescent Photonics

To the

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Pursuant to Phase One STTR FY03 Contract #

F49620-03-C-0053

Research Title	Photonic "Nose" for Chemo- and Bio-Agent Detection: A Novel Surface Enhanced Raman Approach
Reporting Period	September 1,2003 – September 30, 2004
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1. Project Overview

The focus of this phase I STTR is to assess the feasibility of utilizing new photo-generated SERS-active nanostructures, as invented by researchers at JILA, for the construction of viable chemical and biological sensors. This photo-generation process yields "optically written" SERS active sites embedded in a thin polymer film. Critical feasibility concerns are: i) manufacturing reproducibility and tolerances, ii) method of exposing the SERS-sites to the analyte, and iii) demonstration and quantification of the Raman enhancement via recording the spectrum of a known analyte.

1.1. Background

The tremendous potential enabled by SERS based detection has been well demonstrated. First, *ultra-sensitivity* is made possible by remarkable Raman enhancement factors ($>10^{14}$); even delving into the ultimate limit for any sensor, single molecule detection.[1-5] Second, since Raman scattering does not require a fluorescent analyte, SERS offers great *generality* in the range of detectable target molecules.[6] Third, high-resolution Raman spectroscopic "fingerprints" provide *specificity*, i.e., the ability to uniquely identify and discriminate amongst numerous target and non-target contaminant molecules (for larger bio-molecules Raman "tags" can be incorporated into highly specific receptor molecules).[7] In order to capitalize on this potential, however, one requires a synthesis technique and manufacturing protocol for reliable "SERS-active" detection sites.

"SERS-active" sites consist of nano-sized metallic structures (and aggregates of such structures), which, when in proximity to target molecules cause a dramatic increase (can be $>10^{14}$) in the molecules Raman scattering cross-section. Since the first discovery nearly three decades ago,[8] extensive experimental work has identified numerous synthesis techniques[4, 9-24] and ongoing theoretical work has increased our understanding about the origin of the SERS effect.[10, 14, 21, 22] As a point of practical complexity, however, SERS synthesis techniques that are reproducible and amenable to sensor integration, (such as electron-beam micro-lithography, molecular self-assembly, sputtered deposition, and surface roughening), usually exhibit Raman enhancements of only about 10^6. In order to realize the very largest enhancements of 10^{14} one must typically use solvated colloidal silver particles, which hinders reproducibility and system integration. Arguably, it is this compromise between either an amenable form-factor or maximum sensitivity that has prevented SERS based detection systems from realizing their full potential. Obviously, there is a clear need for improved synthesis techniques.

Novel techniques for *in situ* production of SERS-active nanoparticles, via a visible light-induced photogeneration process, have recently been reported. [5, 18-20, 24-27] Most pertinent to this *STTR* is recent work from JILA that demonstrated diffraction-limited photogeneration of SERS-active silver nanoparticles in thin polymer films.[18] The heart of the phase I effort entailed the transfer of this SERS synthesis technology from the JILA laboratories to Vescent Photonics Inc.

2. Key Results and Accomplishments

Critical feasibility concerns, and therefore phase I objectives, are: i) transfer of the technology from an academic lab (JILA) to industry (Vescent), ii) thin film characterization and definition of a manufacturing protocol to provide reproducible SERS-active sites, and iii) demonstration of the Raman enhancement via recording the spectrum of a known analyte. All of the phase I objectives were met, as is summarized below.

- Vescent Photonics worked within the JILA laboratory in order to learn both the synthesis techniques and the metrology methodology associated with photo-generated SERS films. This experience facilitated the design of a Vescent experimental apparatus, which enabled production and analysis of SERS films at the Vescent facility. *This was one of the key objectives for technology transfer from JILA to industry.*
- The polymer films were scrutinized by stylus profilometry, optical profilometry, and atomic force microscopy. The process tolerances for uniform film generation were refined, and most notably, the film thickness was determined to be < 10 nm. This is qualitatively different than previous estimates of ≈100 nm. *Parameter space was explored and a manufacturing protocol was established for reproducible manufacturing of these ultra-thin films.*
- A final objective of this phase I contract was to record the SERS spectrum of a known analyte. Multiple techniques of exposing the SERS-active sites to known analytes were explored. The best results were obtained via doping of analytes into the polymer film, *which yielded spectral features for trans 1-2 bis (3-pyridyl) ethylene (BPE) and toluene. The time resolved nature of these spectra (e.g. blinking) is strongly suggestive of single molecule behavior.*

3. Highlights and Limited Details of Technical Findings

3.1. Technology Transfer: From the JILA Apparatus to a Vescent Apparatus

In the spirit of the *STTR* program, the very first task involved the PI from Vescent Photonics working in the JILA laboratory. This facilitated the technology transfer. Specifically, the components of the experimental arrangement were examined and scrutinized. This "hands-on" experience facilitated the design and construction of a modified optical apparatus for use at Vescent facilities.

3.1.1. The JILA Experimental Apparatus

The JILA optical set-up, used for both photogeneration and Raman scattering, consists of a conventional upright microscope equipped with a dry objective (100x, 0.90 NA). Raman excitation and/or photo-production light is provided by either an argon-ion laser (air-cooled, 488 nm), a Nd:YVO_4 laser (532 nm) or a green Helium-Neon (HeNe) laser (543.5 nm), each coupled into a single-mode optical fiber for spatial filtering. A later modification was to eliminate this single mode fiber, which provided greater optical power through the microsope. This basic

arrangement, along with a picture of the apparatus, is shown in Figure 1-2. The excitation beam is reflected off a dichroic beam splitter, designed to reflect excitation light and transmit red-shifted luminescence, and brought to a nearly diffraction-limited spot (full-width half maximum $\Gamma_{FWHM} \approx 350$ nm). The luminescence from the silver particles is collected by the same objective in epifluorescence geometry. Residual excitation light is suppressed using a combination of two interference filters (each of optical density > 6) and the luminescence is focused onto the aperture of a multimode fiber (100 μm core diameter) acting as a confocal pinhole. In the imaging mode, the fiber is directly coupled to a single-photon counting avalanche photodiode (Perkin model # SPCM-AQR-14-FC 10628) with a dark count level of approximately 90 counts per second (cps). The output from the APD is sent into a multi-function A/D card (National Instruments PCI-MIO-16xE-10) for counting. The driver for the APD is JILA built.

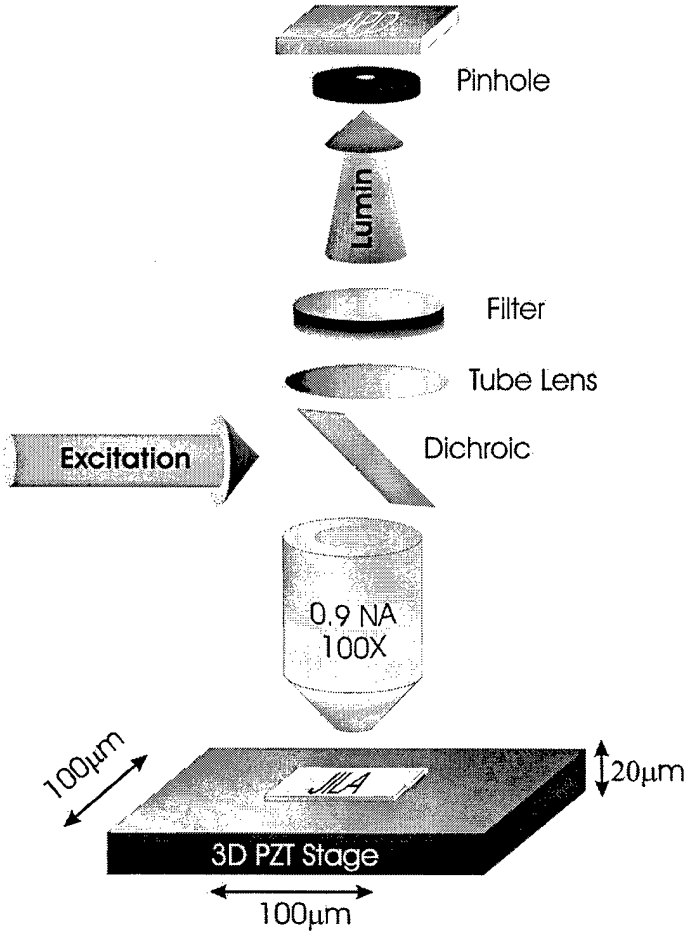


Fig. 2: A schematic representation of the experimental apparatus built at JILA. The same microscope is utilized for both photo-generation and Raman scattering collection. The pinhole is achieved via a multimode fiber (100 μm).

Data can be collected in two modes of operation. In the imaging mode, a two-dimensional image (256 by 256 pixel) of the sample is created by raster-scanning the sample on a computer-

controlled three-axis closed-loop piezoelectric flexure stage. The PZT stage is manufactured by Physik Instruments (PZT Servo Controller E-509.C3 and LVPZT amplifier E-503.00). The silver particles are photochemically generated at arbitrarily chosen points on the thin film. In the spectroscopy mode, the light from the multimode fiber is coupled into a Czerny-Turner type spectrometer (grating 600 lines per mm, f# = 4) and spectrally dispersed onto a liquid nitrogen-cooled CCD detector. Spectra are typically accumulated for 4 s with a spectral resolution of ≈10 cm^{-1}.

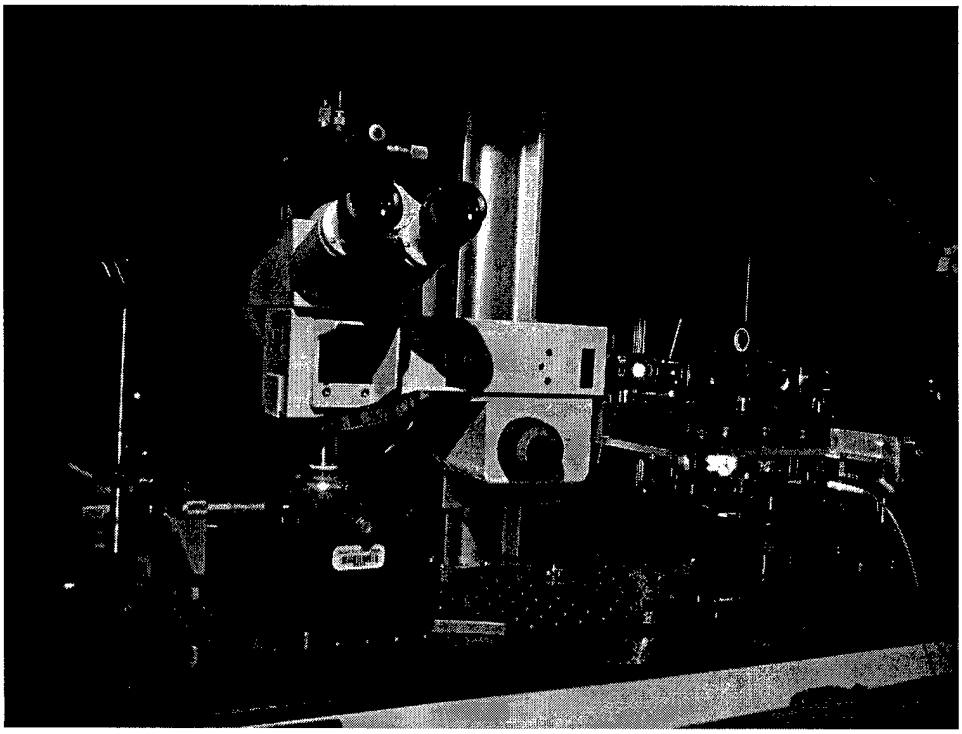


Fig. 3: A picture of the JILA apparatus, with 532 nm generation and excitation light. The spectrometer is located behind the microscope and not visible in this picture.

The microscope eyepieces are useful for initial alignment. Specifically, the back reflected spot is located visually for approximate initial vertical alignment.

3.1.2. Development of the Vescent Apparatus

One of the objectives of the STTR project is to transfer the JILA generated technology into industry. Along these lines, we have designed and built a simplified apparatus for production and analysis of photo-generates SERS films. Shown in Fig. 3 is this apparatus. The entire apparatus is constructed inside a light tight box. A 532 nm beam is expanded (to fully back-fill the asphere), passed through a notch filter (3nm FWHM notch from CVI, PN F10-532.0-4-1.00), then passed through a broad band beam splitter (from CVI, PN BBS-488-694-1025-45), and into a high NA molded glass asphere (focal length of 3mm with an NA of 0.68). The glass coverplate is

held to the surface of a line-tool, which permits alignment of the sample into the focal spot of the asphere. Scattered light, including Raman scattered light, is reflected off the sample, collected by the asphere, and sent back into the broad band beam splitter. Half of the light is sent through a holographic edge filter (from Semrock, model number LP01-432RU-25) which permits only light that is red of the 532 nm line to pass through (within 3 nm). This light is then focused into a multi-mode fiber (600 μm core), which can then be either attached to an optical spectrum analyzer (Ando OSA model # AQ-6315A) or into a photo-multiplier tube (Hamamatsu model number H5784-02). With this apparatus we are able to grow silver nano-particle, SERS-active sites. The growth dynamics are identical to those observed at the JILA facility. Neutral density filters are used to lower the total laser power. Typically, an intensity of 50 kW/cm^2 is used for generation. This corresponds to a total laser power in the microwatt regime, dependant on the focused spot size.

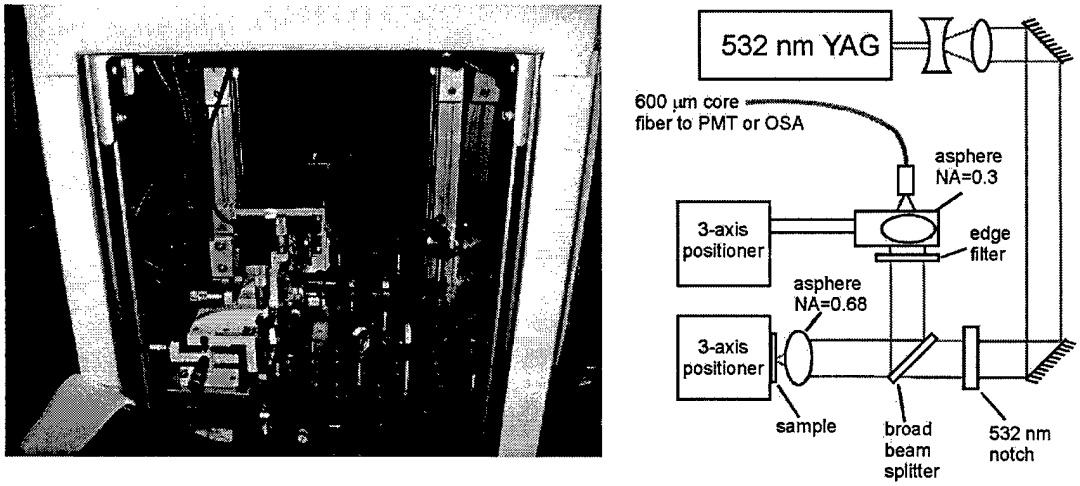


Fig. 3: The simplified experimental apparatus at the Vescent laboratory. Rather than a microscope, a high NA asphere was used. Furthermore, due to phase I budget limitations a line tool micropositioner is used to hold the sample, rather than a piezo translation stage. The intent here is to enable reproducibility tests, not to reproduce the diffraction limited structure generation that is capable at the JILA facility.

Typically, larger features are grown (less tight a focus), since the translation stability for the micrometer positioner is less than for the JILA piezo positioners. This is accomplished via either longer exposure times (up to a few minutes) or increased power.

The relative simplicity of this apparatus is a testament to the overall simplicity of the technique. The entire set-up could be greatly reduced in size. For example, an Ocean Optics Raman spectrometer (with TEC cooled detector array) could be incorporated.

3.2. Characterization and Production of SERS Sites

The adjustable parameter space for this photogeneration technique is sizeable. During the phase I effort the limitations of this parameter space were explored. Specific areas that were explored

were: photogeneration and Raman excitation wavelength, photogeneration optical power densities, and polymer matrix composition and thickness. The requisite photogeneration laser power densities, and their relationship to exposure time, were also explored. Finally, the boundaries and flexibilities with respect to the polymer matrix were explored. Of specific interest were: maximum thickness of the polymer film (if the film can be made thick enough it can serve as an optical waveguide for the raman excitation beam) and the overall effect on the SERS signal.

3.2.1. Thin Film Metrology and Process Refinement

The process of thin film deposition via spin coating results from the balance between solvent-substrate affinities, evaporation rates, and centrifugal forces. The polystyrene mixture/glass substrate affinity is very low, as observed by beading up on the surface (or lack of wetting when dropped on tilted substrates). Under conventional industrial spin-process methods this would result in poor quality films and is typically avoided. With this caveat in mind, the polystyrene films were carefully characterized by three metrology techniques: atomic force microscopy (Digital Instruments Dimension 3100 at JILA), optical profilometry (Wyko Model NT2000 at the CAPT center via Vescent), and stylus profilometry (Dektak Model 200Vsi at the CAPT center via Vescent). As shown in Fig. 3 and 4, uniform films can be generated, however, with a remarkably small thickness (only 3-7 nm). Furthermore, dependent on spin coat time and spin speed, one can easily generate highly non-uniform films. For example, if the spin is finished prior to full evaporation of the solvent then pooling can lead to large "islands" of polystyrene. Such non-uniform films can manifest themselves as non-reproducible nano-structure growth kinetics, and non-reproducible SERS. Refining the manufacturing protocol for improved reproducibility was a significant achievement in this first phase.

With the thin film metrology tools in place, the manufacturing tolerances for these films can be explored. As we discovered, such extremely thin films are highly sensitive to production variants. For example, doping of pyridine into the film destroyed the film quality. Likewise, alternate polymers (such as PVA with water as a solvent) provide less uniform films. Additionally, the film quality is highly sensitive to the cleanliness of the substrate. Ozone cleaning is necessary to provide uniform films. Another issues was sufficient spin-coat time. If the solution was not spun for sufficiently long (>20 seconds) then non-uniform films would result. The optimum recipe as of this date is: 10 mg of polystyrene, 20.74 mg of silverperchlorate, dissolved in 10 ml of toluene. This is spun coated onto a float glass cover slip that has been ozone cleaned. The spin speed is 3000 rpm, and one 20 ul drop is applied and allowed to spin for at least 20 seconds.

This process produces reproducible and uniform films, with a thickness of < 10 nm. This thickness prohibits the use of the film itself as a waveguide for the excitation light, however it does present other interesting characteristics.

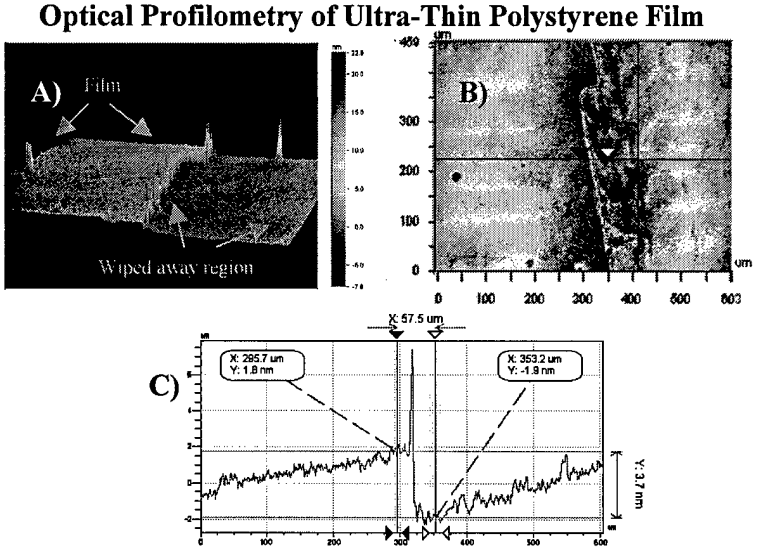


Fig. 3: Optical profilometer scan (depth resolution 3 Angstroms) over the edge of an ultra-thin polystyrene film, such as will be used as a matrix for embedding photo-generated, SERS active silver nano-clusters. Wiping away the film with lens tissue soaked in toluene created the edge. Scratching away the film with wooden toothpicks, dipping the film in solvents, wiping with q-tips, and scratching with razor blades also created edges. All techniques yielded similar height results. Figure A shows a 3-D profile, while Figure B shows a surface plot. The horizontal red line in Fig. B is shown as a lineplot in Fig C. For this case the film height is only 3.7 nm. The large spike directly at the interface is due to material buildup along the wipe line.

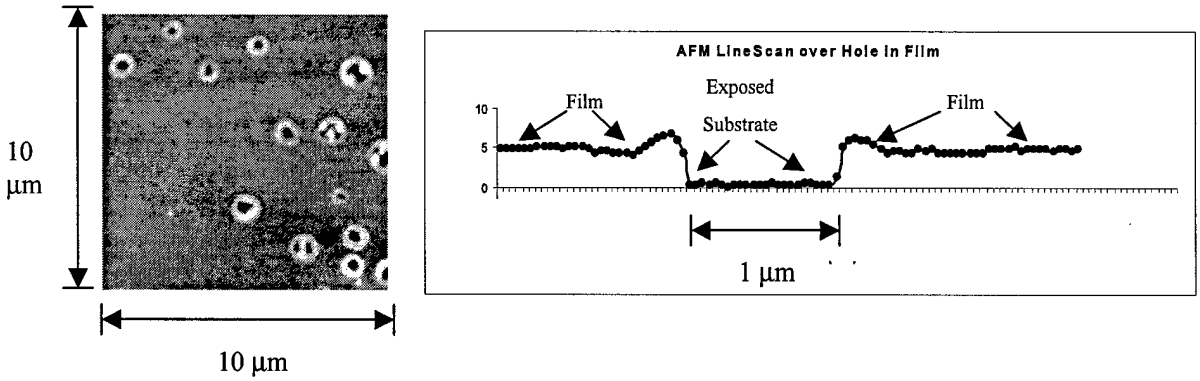


Fig. 4: Atomic Force Microscope scan of the edge interfacial region. The solvent (toluene) has a low affinity for the substrate (Corning float glass 1737), and therefore droplets create holes in the film, with polystyrine build-up surrounding the holes. AFM linescans over these holes provide a measure of the films thickness. In this case the film is only ≈5 nm thick.

The ultimate goal of this STTR is a viable sensor that utilizes the extreme sensitivity realized via surface enhanced Raman spectroscopy. One of the prominent hurdles in realizing this goal is construction of the SERS nano-structures in a form-factor, or platform, which enables simultaneous exposure to the analyte and Raman probing. The silver nano-clusters have been determined to be large clusters of 1 nm size silver particles, and therefore the total cluster is expected to protrude above the < 10 nm film. Shown in Fig. 5 are luminescence and AFM scans of the same sample. The photo-generated pattern that is clearly visible in the luminescence scan can also be seen in the AFM scan. This bodes well for exposing the analyte to the nano-cluster from above the film. In other words, this technique naturally provides SERS active nano-clusters that are already embedded in a thin film in such a way that could be amenable to a sensor structure.

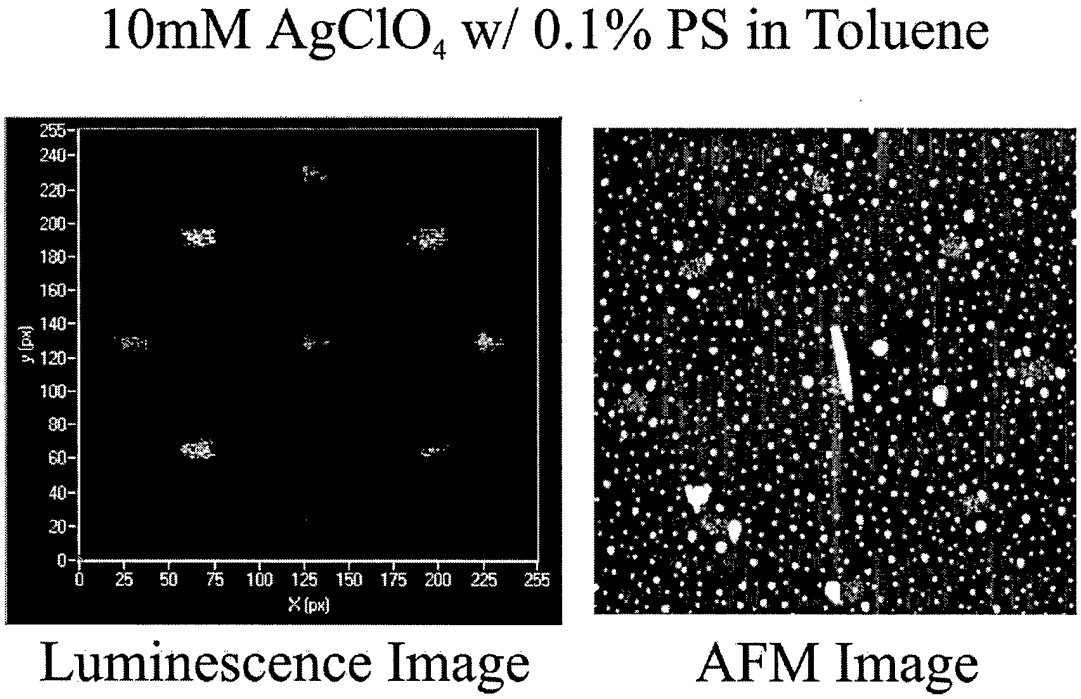


Fig. 5: Optical luminescence scan over a SERS written image (left), this was performed at the JILA apparatus. The right side of the figure depicts the same sample but scanned with an AFM. The same image is visible, indicating that the SERS nanostructures are protruding above the polystyrene film.

3.2.2. Characterization of SERS sites

The photo-generated silver nano-clusters are created while embedded in a polymer matrix. The presence of this matrix could hamper analyte interaction. We therefore attempted to wash away the polymer matrix and then looked to see if the the SERS-active sites were still present on the substrate. The wash was prefomed via removing the sample from the instrument and then applying first toluene and then water. The toluene is used to remove the polystyrene and first amount of silver percholrate. The water is used to remove residual toluene and silver percholrate. Both solvents were applied to the sample via a spin process. Specifically, 1 ml of solvent is applied to the sample under a 3000 rpm spin. The spin is continued for > 1 minute to allow for full solvent removal. Shown in Figure 6 is a photoluminescence scan over a SERS pattern that has been washed. As can be seen, the SERS sites are still present, and therefore must be bonded to the substrate.

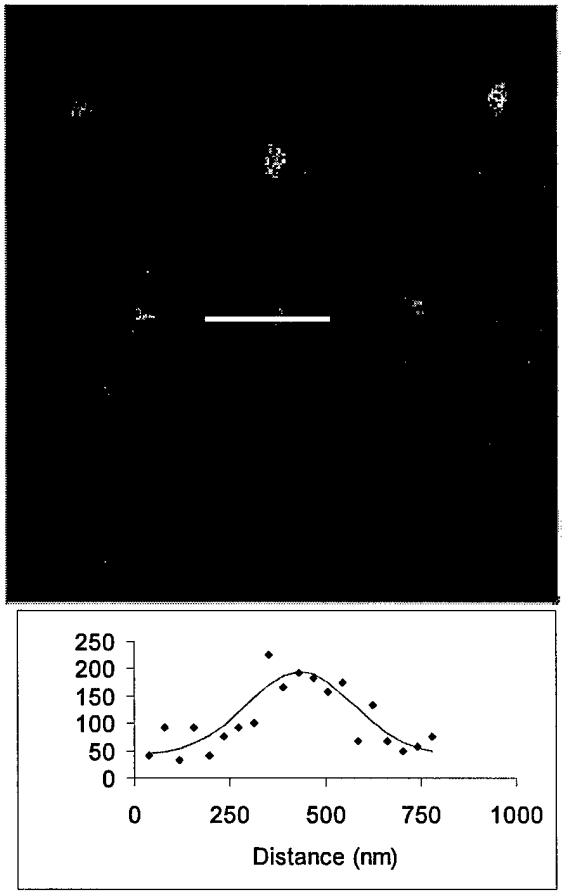


Fig. 6: Optical luminescence scan over a SERS written image that has then had the polymer matrix removed via a wash. The bottom panel shows a line scan over the central spot (scan region shown as the white line in the top figure). The feature size is 685 ±85 nm.

Shown in Figure 7 is an AFM scan over a SERS pattern that has been washed. This is the same sample as from Figure 6. As can be seen, the SERS sites are still present, and therefore must be bonded to the substrate.

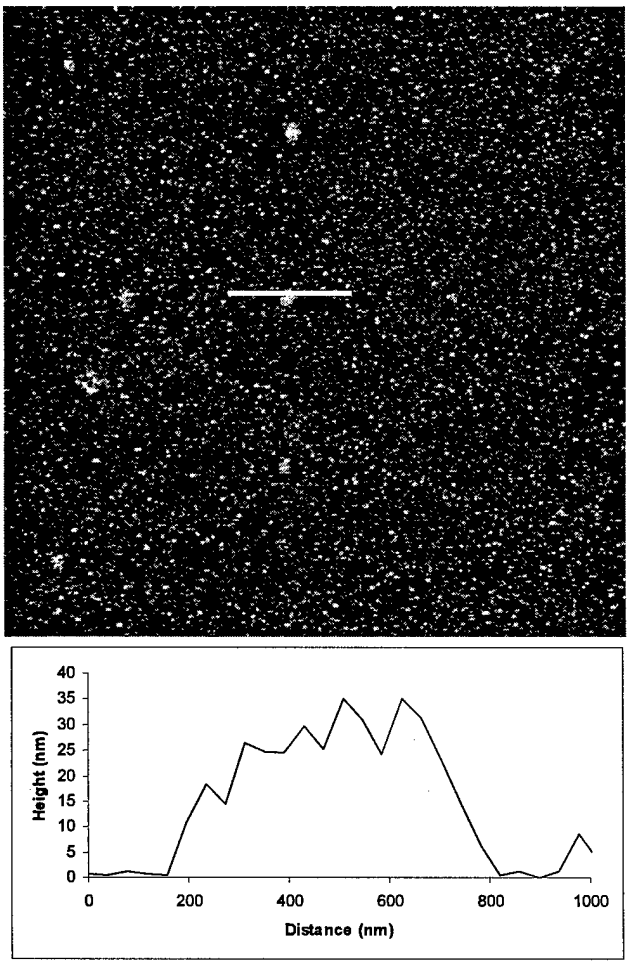


Fig. 7: AFM scan over a SERS written image that has then had the polymer matrix removed via a wash. The bottom panel shows a line scan over the central spot (scan region shown as the white line in the top figure). This is the same sample as in Figure 6. The feature size is 435 ±40 nm.

It was postulated previously that the SERS active sites consisted of aggregates of silver nano-particles. With these washed films, and the resolution enables by the AFM, we can directly probe for this. Shown in Figure 8 is a close up scan of the central feature of Figure 7. The silver aggregate can readily be seen.

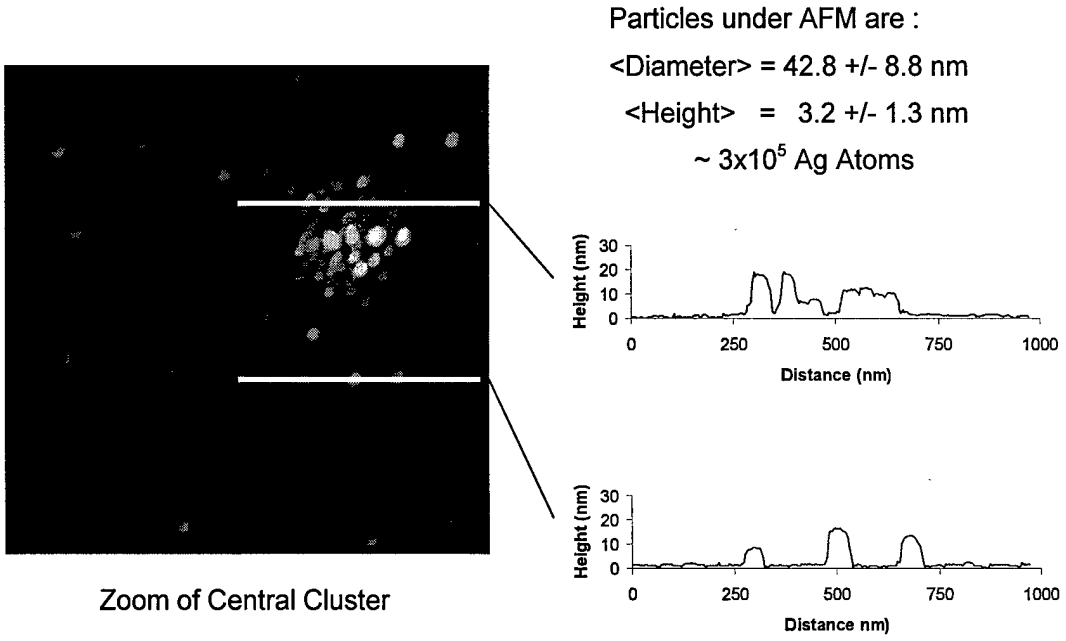


Fig. 8: Close up AFM scan over a SERS written feature that has then had the polymer matrix removed via a wash. The right panels show line scans over the central spot (scan region shown as the white line in the left figure). This is the same sample as in Figure 6 and 7.

In addition to characterizing the thin film and the silver nano-clusters, we also characterized the growth parameters. For example, shown in Figure 9 is a dependence of the growth on illumination power. As can be seen in the figure, the growth does saturate at higher powers, and therefore one can only gain so much with higher power.

In addition to producing uniform films (which is essential for reproducible growth), one must also be careful to produce robust growth patterns. For example, we experimented with growing SERS clusters on Si substrates. Not only were the growth kinetics highly variable (sometimes nothing grew), but there seemed to be spontaneous growth in regions that were not illuminated. At this time this is not well understood. It could either be thermal growth, or a high surface mobility. Nevertheless, the recipe which we identified provided reliable results.

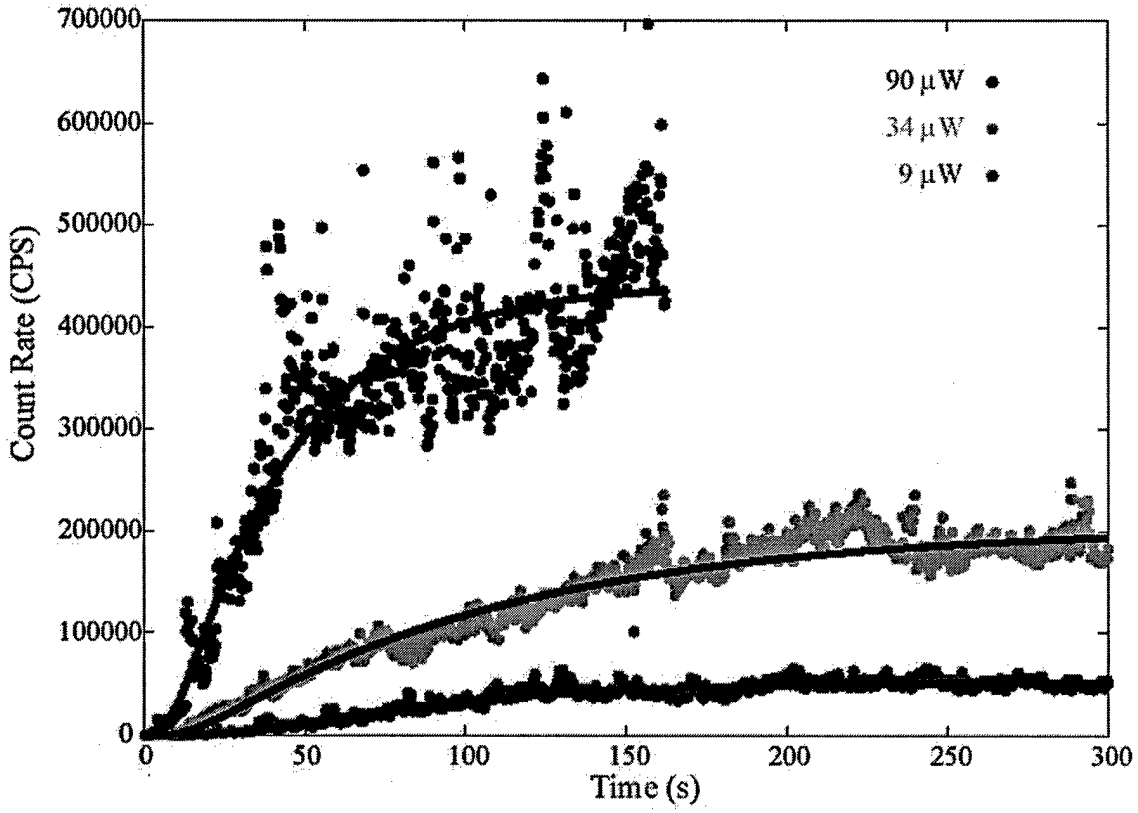


Fig. 9: Investigation of power dependence for silver nano-cluster growth.

3.3. The Search for a Known Analyte

The search for a SERS spectral signature from a known analyte is hindered by two aspects. First, there is the challenge of creating intimate analyte-nanocluster interaction. This is required for SERS enhancement. For example, early in the project we attempted to dope pyridine into the polymer matrix, in hopes that pyridine would be intimately held next to the nano-structure. Unfortunately, the presence of pyridine disrupts the spin coating process of the polystyrene. Specifically, rather than a uniform film, islands or clumps of polystyrene are left behind. This can be observed as a "milky" layer on the glass substrate. Second, the very nature of SERS can alter or modifies the spectral patterns. For example, a solution of 1% by volume thiophenol (or 0.01 mM) in a 10 mM solution of silver perchlorate in toluene with 1 mg polystyrene per 1 ml toluene was spun coated onto the glass substrate (Corning Float Glass). The photoreduction of the silver (the write process) proceeded much more rapidly, and the Raman scattering (the read

process) was >100 times more intense. Unfortunately, the spectrum did not show characteristic thiophenol peaks. This may be due to the thiophenol being attached to the Ag cluster via the S and losing the H, which would eliminate the "fingerprint" S-H peak.

Despite these challenges, we continued to search for a technique to observe spectra from a known chromaphore. In the rest of this section we will describe two example approach's that we utilized. First, however, it is worth discussing the nature of the spectral signatures that we observe.

3.3.1. The "Flavor" of photo-generated SERS Spectra

Most SERS work is plagued by strong Raman transitions appearing in the range of 800 cm^{-1} to 1800 cm^{-1}, often attributed to impurities or so-called "carbonaceous contaminations". Despite the frequent observation of these "carbonaceous" Raman bands in SERS, the origin and abundance of graphite-like Raman bands is still unclear. In our spectra we also observe these broad features. Shown in Figure 10 are time sequence data showing this broad background that is always present

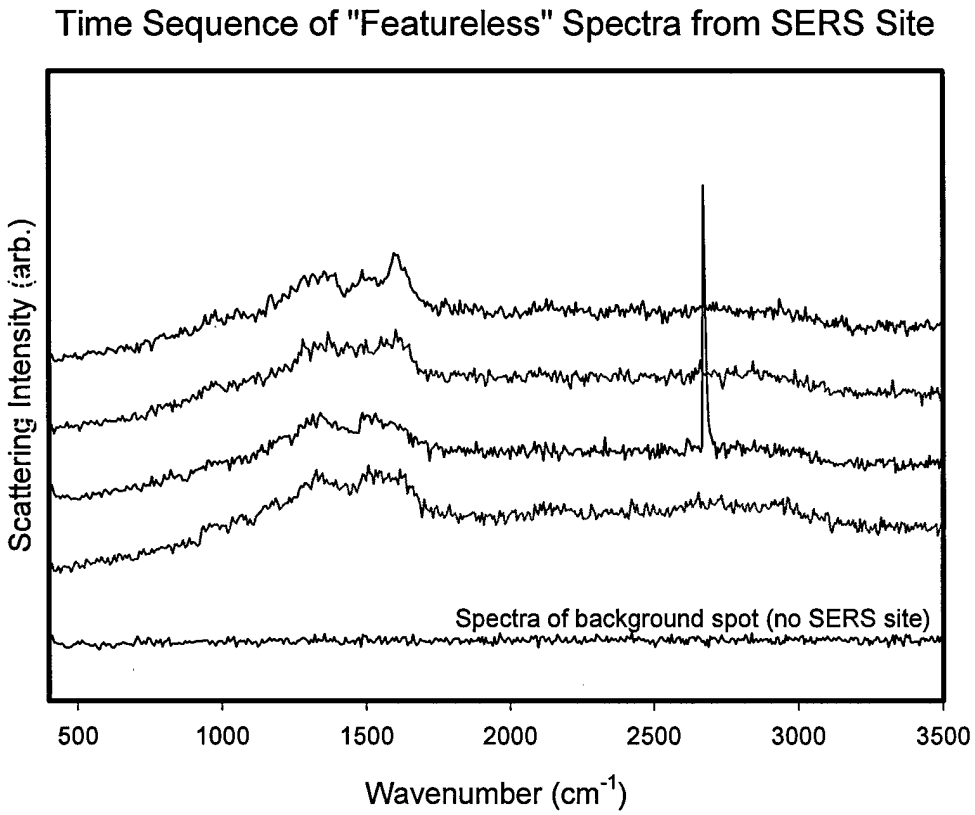


Fig. 10: Examples of spectra taken from a photogenerated SERS site. These example spectra show no resolved spectral features, but rather display the broad background that is pandemic to all of our SERS spectra.

in our spectra. In the plot each spectra was taken five seconds apart. They are displaced in the vertical dimension for visual clarity. Shown in the bottom trace is a background spectra. Specifically, the background spectra were taken in a region where no nano-clusters were grown. The large spike in the blue trace is not a feature, but rather due to a cosmic ray impinging on the CCD. In this plot, as in all of the plots for this section, the excitation and growth light was a 532 nm YAG laser. All of the films were produced via our best determined recipe, i.e., silver perchlorate and polystyrene dissolved in toluene.

Of course, sometimes well resolved spectral features are observed. This is displayed in Figure 11. Here, the intensity of the feature is considerably larger (approximately a factor of five) than the feature plotted in Figure 10. As can be seen in figure 11, again the broad features are present-however, for the spectra recorded at 15 seconds (really the data was integrated from 10 to 15 seconds) there are definite and well resolved spectral features. It is as if the spectra just "blinked" on, and then for the next set of data it "blinked" off. This is not unexpected for single molecules, and therefore suggestive that we are recording the spectra from a single molecule.

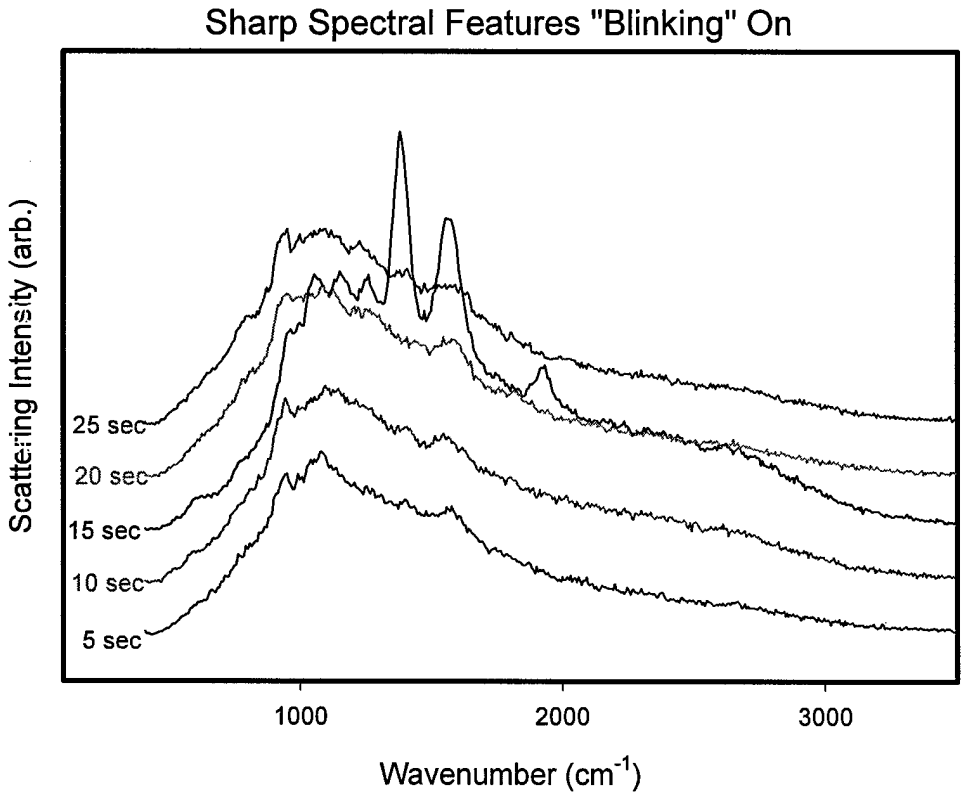


Fig. 11: Examples of spectra taken from a photogenerated SERS site. These example spectra show no resolved spectral features except for at one time trace (15 seconds). This blinking behavior (spectra turning on and off) is indicative of single molecule. In this sample there were no known dopants, other than the toluene and the polystyrene. The identity of this sharp chromaphore is not known.

In order to have a useful sensor one must not only observe the spectra, even if it is from a single molecule, but one must also be able to use the spectra to identify the analyte. We therefore attempted to apply a known analyte and then search for that analytes spectra. As discussed briefly above, we attempted to use pyridine and thiophenol, yet without much success. It would be tempting to use a dye molecule such as a Rhodamine, however, the fluorescence would dominate and obfuscate the desired Raman data. We therefore chose to utilize a salt, trans 1-2 bis(4-pyridyl)ethylene, otherwise known as BPE ($C_{12}H_{10}N_2$). This analyte is desirable because it can be solvated into methanol and toluene, and the Raman spectrum has prominent C-N peaks just above 1600 cm^{-1}.

Two approaches were taken to try and observe the SERS spectra of BPE. In both cases SERS active sites were photogenerated with 532 nm light. For the first approach we then washed away the polymer film and spun coat on a solution containing BPE. For the second approach we doped a small amount of BPE directly into the film.

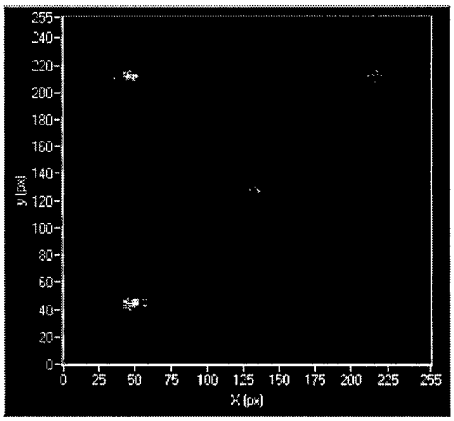
3.3.2. Washed Film Approach

An "X" pattern of photogenerated SERS sites was created. A photoluminescence scan of this pattern is shown in the top trace of Figure 12. Spectra were recorded at several of the SERS features, with the typical results, such as shown in Figure 11. Prior to writing the SERS features, a cross was drawn on the back of the coverslip with a marker. This pattern was grown directly in the vertex of this cross, which enabled removal and return of the sample. By locating the vertex in the microscope the SERS pattern could then be located again. In Figure 12 the scans are 15x15 microns.

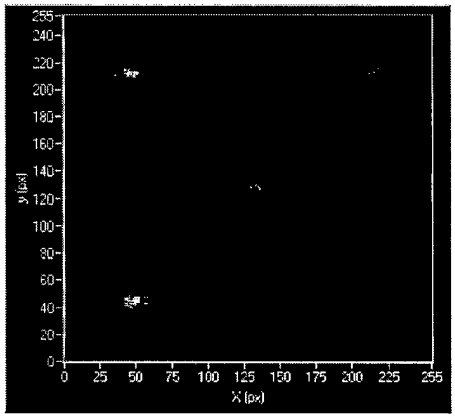
After generation of the pattern the slide was removed and then washed. First, 1 ml of toluene was applied to the sample as it was spun at 3000 rpm. This step served to remove the polystyrene and much of the silver perchlorate. Second, 1 ml of water was dropped onto the spinning sample. This helps remove the rest of the silver perchlorate and any residual toluene. The sample was then placed back into the system and the SERS pattern located. The photoluminescence scan of the washed sample is shown in the central panel of Figure 12. The total integrated intensity of the central Raman feature is reduced by approximately a factor of two. The spectra of this Raman light was recorded. After the wash the spectra was both reduced in size and it generally did not contain any sharp features. It was just the broad background. One possibility is that the sharp features (such as shown in Figure 11) are due to either toluene or the polystyrene. The hope would then be that application of an analyte would result in well resolved spectral features.

The bottom panel of Figure 12 shows the photoluminescence scan of the SERS pattern after a dilute sample of BPE was applied. Specifically, the sample was removed and a 25 microliter drop of 1 millimolar BPE in toluene was applied to the spinning sample. The photoluminescence image was then obtained. The integrated intensity of the center feature increased back up to approximately the pre-wash level. The spectrum of this central feature was then recorded. The spectrum is plotted in Figure 13.

Pattern Before Wash
Integrated Intensity of
Center Feature:
5403 photons



Pattern after wash
Integrated Intensity of
Center Feature:
2912 photons



Pattern after wash
and after analyte exposure
Integrated Intensity of
Center Feature:
4968 photons

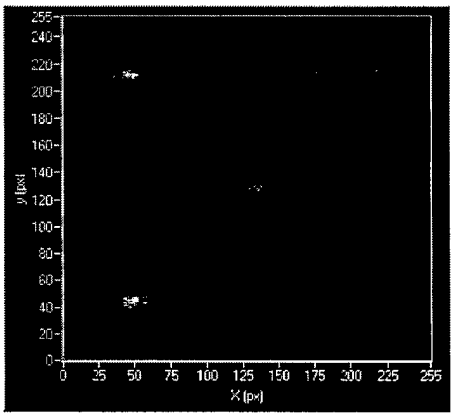


Fig. 12: A photogenerated SERS pattern during different stages of the "wash experiment". The top panel shows the original image. The middle panel shows the pattern after removal of the polymer matrix via a wash. The bottom panel shows the same pattern, but after spin coating on a dilute sample of BPE.

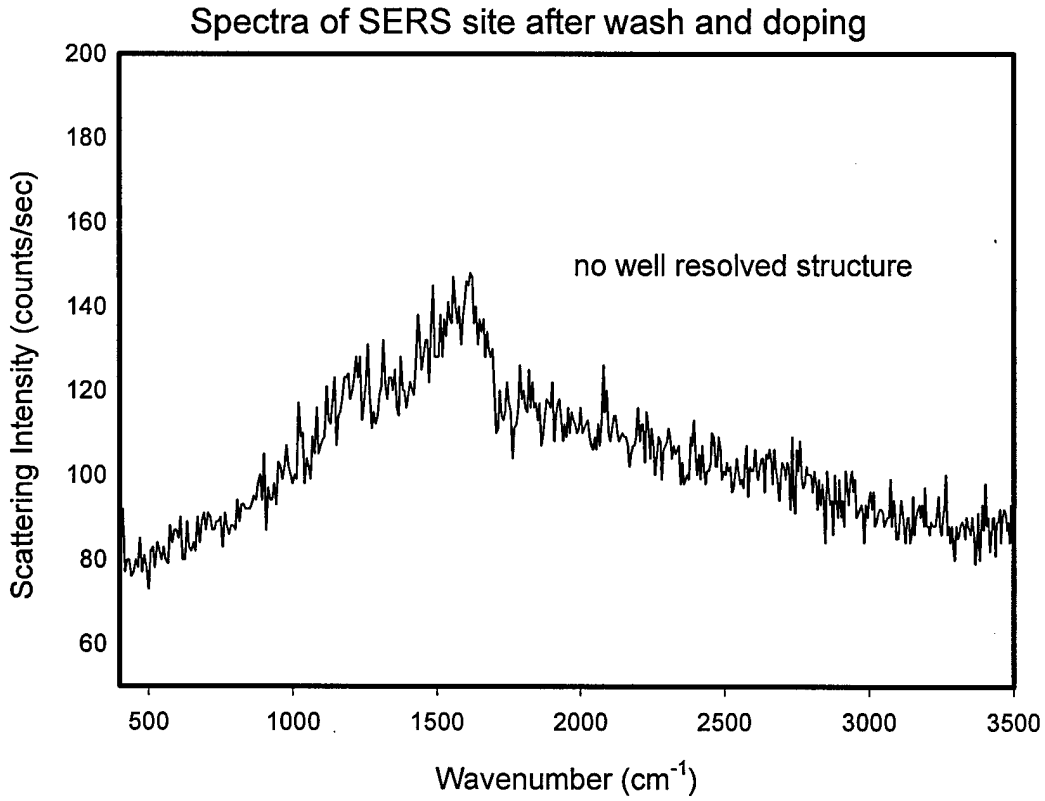


Fig. 13: Spectrum of the central feature from the bottom panel of Figure 12. The hope was that this might display well resolved BPE peaks. Unfortunately, only the standard broad background was recorded.

As can be seen from Figure 13, the spectrum does not display any well resolved spectral features. This spectrum was recorded multiple times, all with the same result. Furthermore, this whole process was conducted multiple time, with varying parameters. Again, after the wash only broad spectral features are observed. It seems that in order to observe strong Raman enhancement the chromaphore must be embedded within the polymer matrix.

3.3.3. Chromaphore Doped into the Polymer Matrix

Rather than wash the polymer away and then expose the analyte to the exposed silver nano-clusters, one may directly dope the analyte into the matrix. In a sense this is performed everytime we grow a nano-cluster. Specifically, small amounts of toluene do not evaporate during the spin process, but rather are trapped within the matrix. These toluene molecules could be positioned close to a nanocluster, and these could explain the sharp features that are sporadically observed in the spectra. Before doping an additional chromaphore, we therefore recorded the traditional Raman spectrum of bulk toluene. Toluene was applied to a well slide and then placed in the same

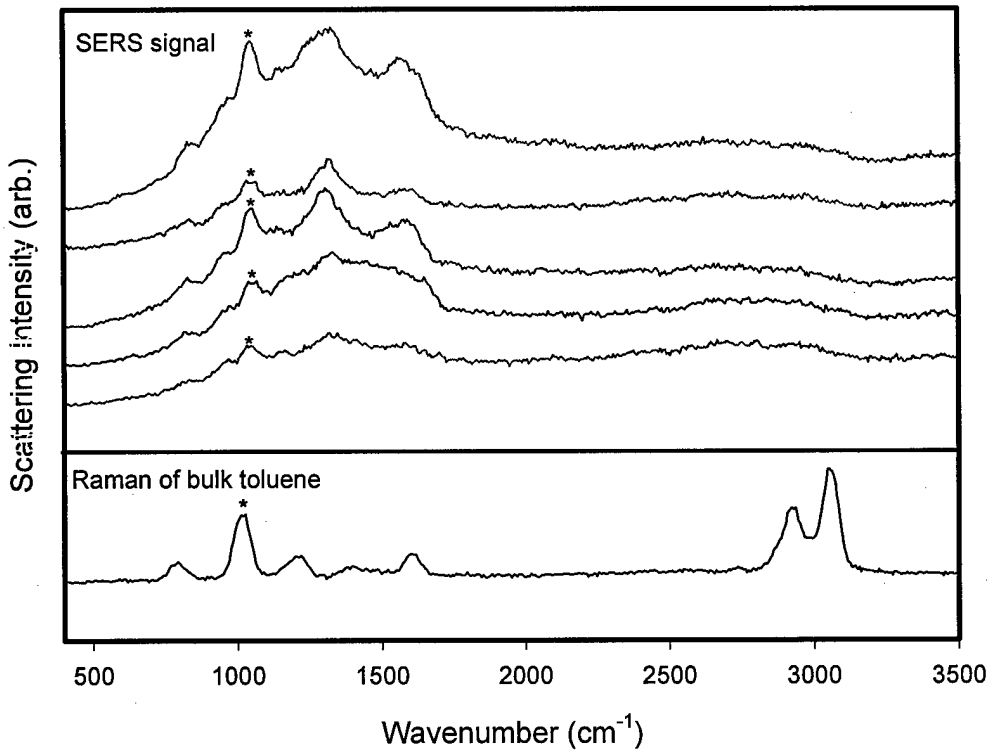


Fig. 14: The bottom trace is the Raman spectrum of bulk toluene. The top traces are SERS spectra from a photogenerated SERS site. These spectra display the typical broad spectral features, but they also display the sporadic "high resolution" peaks. Identified with an asterix is a feature from the bulk toluene that could be present in the SERS spectra. This is consistent with the toluene being the chromaphore. Note, the C-H stretches at 3000 cm^{-1} are missing in the SERS spectrum. This is not unusual, since the act of adhesion to the nano-cluster can quench the C-H motion. Indeed, the broad features just to the red of the C-H stretches could be the remnant of a highly quenched C-H stretch.

apparatus. The Raman spectrum of bulk toluene is shown in the bottom trace of Figure 14. The sequence of SERS spectra represent a time sequence of five, five second scans. In this case,

rather than blinking on and off the spectra appears to more gradually be growing and subsiding. Some immediate observations are that the C-H stretches are not present in the SERS signal. This could be due to a similar process that quenched the S-H stretches in thiophenol. Notably, the strong peak at 1000 cm^{-1} does appear to be present in the SERS spectra. This is by no means unequivocal proof of single molecule toluene spectra, but it is suggestive. One problem with all single molecule approaches is that the spectral signatures are likely dependent on that molecules orientation with respect to the nano-cluster. This could impact both the position of spectral features, and their relative intensity. This in turn may limit specificity.

In an attempt to test the specificity, the analyte BPE was doped into the polymer matrix. Specifically, 0.05 milimolar of BPE was added to the polystyrene toluene mix. Similarly, the Raman spectrum of bulk BPE was recorded. This was accomplished by spin coating on a thin

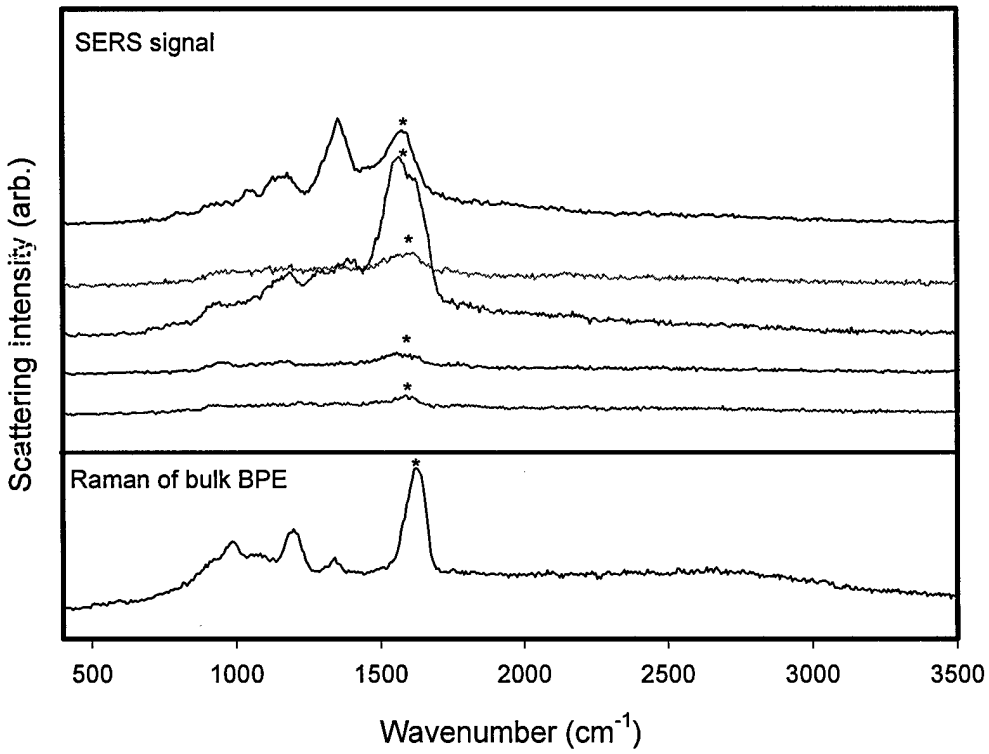


Fig. 15: The bottom trace is the Raman spectrum of bulk BPE. The top traces are SERS spectra from a photogenerated SERS site. These spectra display the typical broad spectral features, but they also display the sporadic "high resolution" peaks. Identified with an asterix is a feature from the bulk BPE that could be present in the SERS spectra. This is consistent with the BPE being the chromaphore. Note, the C-H stretches at 3000 cm^{-1} are greatly reduced in the bulk spectra. Likewise, the C-H stretch hump observed in the spectra of Figure 14 is also absent.

BPE film to a blank coverslip. This Raman spectrum is plotted in the bottom trace of Figure 15. The top traces of Figure 15 show a time sequence of SERS spectra. Evident in this example scan

is a large feature near 1600 cm^{-1} that "blink" on and off. Again, this is not unequivocal proof of single molecule BPE spectra, but it is suggestive.

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